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Taxonomical studies of four new Aseptate Gregarine parasites belonging to the Genus *Monocystis* Stein, 1848 (Protozoa: Apicomplexa: Sporozoa) from an Oligochaete Host, *Eutyphoeus orientalis* (Annelida: Oligochaeta) of West Bengal, India

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Abstract

Surveys of acephaline gregarines in the oligochaetes from various districts of West Bengal, India revealed the occurrence of four new species of *Monocystis* Stein, 1848, *Monocystis eutyphae* sp. nov., *Monocystis indicus* sp. nov., *Monocystis satoi* sp. nov. and *Monocystis csabai* sp. nov. in the seminal vesicles of the earthworm, *Eutyphoeus orientalis*. Trophozoites of *Monocystis eutyphae* sp. nov. fusiform, measuring 100.7-107.8 (104.83±2.02) µm x 51.93-59.67(3.13±0.69) µm. Nucleus rounded, measuring 10.5-11.0 (9.0±1.05) µm. Gametocysts ovoid with two unequal gametocytes, measures 89.50-96.13(93.04±2.81) µm x 67.40-78.45(72.37±4.35) µm. Oocysts biconical, measuring 13.25-15.47(14.30±0.91) µm x 9.94-13.26 (11.70±1.40) µm. Gamonts of *Monocystis indicus* sp. nov. oval shaped, measures 89.61-111.6 (102.95±9.69) µm x 59.29-70.72(66.20±4.33) µm. Gametocysts rounded. Oocysts biconical, measuring 10.55-11.00 (10.77± 0.46) µm x 7.00-8.50 (6.0± 0.50) µm. Syzygy present. Trophozoites of *Monocystis satoi* sp.nov. flat and oval shaped, measures 96.55-125.00(15.25±8.25) µm x 50.28-62.50(59.22±5.00) µm. Nucleus rounded, measuring 12.35-14.50(13.75±1.00) µm. Syzygy present. Gametocysts rounded contains two unequal gametocytes having 70.00-75.00(72.00±4.32) µm in diameter. Oocysts biconical, measuring 15.17-18.17 (16.70±1,35) µm x 9.75-11.25(10.00±1.00) µm. Trophozoites of *Monocystis csabai* sp. nov. elongated, irregular flat like, measures 132.0-160.0(145.0±4.50) µm x 30.0-35.0(32.2±2.50) µm. Nucleus rounded, measuring 15.5-20.0(17.2±1.5) µm. Gametocysts almost rounded, measuring 60.0-70.0(65.0±2.50) µm. Oocysts biconical, measures 9.0-12.5(11.0±1.5) µm x 6.5-7.5(7.0±0.96) µm.

Keywords: Apicomplexa, Eugregarinida, Gregarine, Oligochaetes, Seminal Vesicles

Introduction

Gregarines are mainly coelozoic endoparasites. The protozoan parasite belongs to phylum Apicomplexa, order Eugregarinorida (Leger, 1900) under family Monocystidae. Approximately, two hundred fifty genera and one thousand six hundred fifty species of gregarines have so far been described till date (Levine, 1976, 1977a, b, c, d and 1988; Clopton, 2000; Hausmann *et al*., 2003).

The gregarines group represent two form acephaline and cephaline. Acephaline gregarine infects oligochaete host. Cephaline gregarine tends to harbour mainly arthropods and insects. Acephaline gregarines are characterised by undevided septum whereas cephaline gregarine bears septum. More than 350 earthworm species have so far been examined for exploration of endoparasitic acephaline gregarines fauna in India.

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Till date, only 91 species of aseptate gregarines under the genus *Monocystis* have so far been recorded throughout the world (Von stein, 1848; Labbe, 1899; Cuenot, 1901; Drzhevetskii, 1907; Hesse, 1909; Boldt, 1910; Mulsow, 1911; Cognetii de martiis, 1911, Pinto, 1918; Voss,1921; Ghosh, 1923; Berlin, 1923,1924; Bhatia and Chatterjee, 1925; Hahn, 1928; Sciacchitano, 1931; Tuzet and Loubatieres, 1946,1948; Loubatieres, 1955; Tuzet and Zuber Vogeli, 1955, 1956; Meire, 1956; Boisson, 1957; Rees, 1961; Bereczky, 1967; Segun, 1968, 1978; Giere, 1971; Righi, 1974; Levine, 1977 Subbarao *et al*., 1979; Pradhan and Dasgupta, 1982; Bandyopadhyay *et al*., 2001; Bandyopadhyay and Biswas, 2002; Bandyopadhyay and Mitra, 2005; Bandyopadhyay *et al*., 2005, 2006a, b, c, d, 2007, 2008, 2009a and b; Bhowmik *et al*., 2011; Sarkar and Bandyopadhyay, 2011, 2013 and 2014; Mallik and Bandyopadhyay, 2017).

The biodiversity survey of gregarines from earthworm in various districts of West Bengal revealed four new species of the genus *Monocystis*. This communication contains taxonomic description of four new species of *Monocystis* found in the seminal vesicles of earthworm, *Eutyphoeus orientalis*. Morphometric comparison with closely related species and its taxonomic summery have also been provided here.

Material and Methods

Materials used for the present communication were collected from the district of North and South 24 Parganas of West Bengal. Collected earthworms were brought alive at the parasitology laboratory of the University of Kalyani, Kalyani. The type host of the four new species have been deposited in Zoological Survey of India, Kolkata, West Bengal, India. Earthworms were dissected and removed their seminal vesicles carefully. On clean glass, with a drop of 0.62 % (w/v) NaCl solution content of seminal vesicles were placed. On a slide a thin film of the seminal fluid was drawn out and covered with a cover slip for examination of live protozoans under the phase contrast microscope. Then semidried the contents of seminal vesicles and fixed in saturated Schaudin's fluid for 20 min. In 70% ethyl alcohol, the fixed smears were stored for removal of mercuric chloride. The slides were then passed through a descending series of alcohols (5 min each) and stored in distilled water. These were transferred to a 3% iron alum solution and stained with Heidenhain's haematoxylin solution (20 min). Differentiation was done with 1 % iron alum solution. The slides were then washed thoroughly, dehydrated in an ascending series of alcohol, cleared in xylene and mounted in DPX. Drawings of the different stages of gregarines were made using a camera lucida attached to the microscope. Photomicrographs were taken using an Olympus light microscope (model CH-2) and Olympus digital camera (model CX41). All measurements have been taken with a calibrated ocular micrometer (µm).

Systematic Account

Phylum APICOMPLEXA Levine, 1988
Class SPOROZOA Leuckart, 1879
Order EUGREGARINIDA Leger, 1900
Family MONOCYSTIDAE Butschli, 1882
Subfamily MONOCYSTINAE Bhatia, 1930
Genus ***Monocystis*** Stein, 1848

Results and Discussion

During the present study four new species belonging to the genus *Monocystis* Stein, 1848 have so far been found from the seminal vesicles of *Eutyphoeus orientalis*. Descriptions of these aseptate gregarines are provided below:

Monocystis eutyphae sp. nov. Figures 1(A-D), 2(AD) Table A & Table 1

Description

In *Monocystis eutyphae* sp. nov. gamonts are fusiform, anterior portion is wider than the posterior end. The anterior end contains mucron. Nucleus is rounded, situated in the middle portion of the body. The pellicle is thin. Presence of highly granulated endosarc contains paraglycogen granules. Gametocyst ovoid with two unequal gametocytes. Oocysts are biconical.

Gamont measures 100.7 - 107.8 (104.83 ± 2.02) µm in length and width of the anterior part of gamont is 51.93 - 59.67 (55.68±3.13) µm and Posterior part is 20.99-23.86 (22.69 ± 1.08) µm. Diameter of nucleus is 10.5-11.0 (9.0 ± 1.05). Gametocyst measures 89.50-96.13(93.04 ± 2.81) µm in length and 67.40 - 78.45(72.37 ± 4.35) µm in width. Length of the oocyst measuring 13.25-15.47(14.30 ± 0.91) µm and width measuring 9.94-13.26 (11.70 ± 1.40) µm.

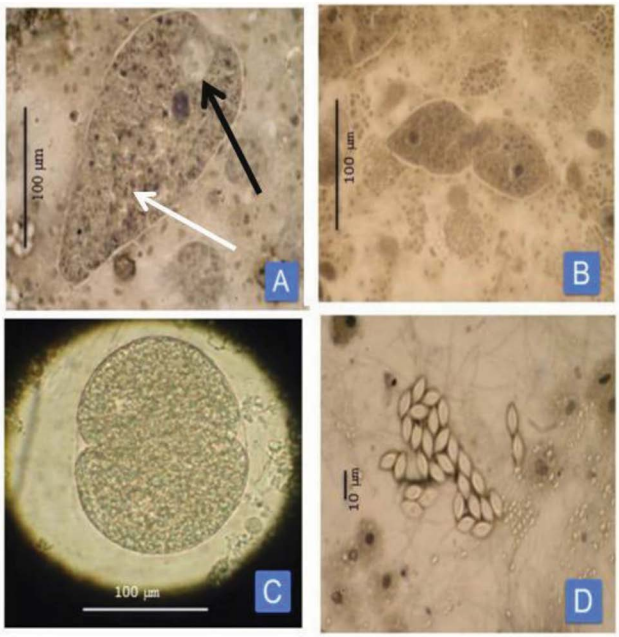

Figure 1. Photomicrographs of different stages of the life history of *Monocystis eutyphae* sp. nov. obtained from the seminal vesicles of earthworm *Eutyphoeus orientalis*, **A.** Trophozoite(Black arrow - mucron); (white arrow - granulated endosarc). **B.** Syzygy. **C.** Gametocyst. **D.** Oocyst . Scales: A - C = 100µm; D = 10µm

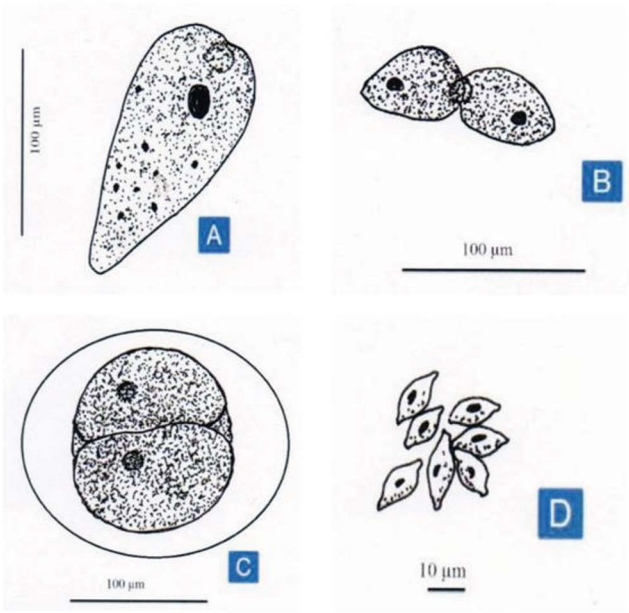

Figure 2. *Monocystis eutyphae* sp. nov. (camera lucida drawings of different stages of life cycle) **A.** Trophozoite. **B.** Syzygy. **C.** Gametocyst. **D.** Oocyst. Scales: A-C = 100µm; D=10µm.

Taxonomic Summary

Type host: *Eutyphoeus orientalis*.
Site of infection: Seminal vesicles.
Type locality: Ashoknagar, North 24 Parganas (23°15'2" N- 89°5'E), West Bengal, India.
Prevalence: 20 out of 40 host earthworms (50%) were infested with the parasite.

Material deposited

Holotype: PR/MC/01/12 has been deposited at the Parasitology Laboratory, Department of Zoology, University of Kalyani 741235, and West Bengal, India.

Paratype: The paratype has been deposited in the Harold W Manter, Laboratory of Parasitology, Systematic Research Collections, University of Nebraska State Museum, W436, Nebraska Hall, Lincoln, Nebraska 68588-0514 USA,(Accession no. P-2014-002; Reg.no. HWML 49901).

Etymology: The species name has been derived from the generic name of the host specimen, *Eutyphoeus orientalis.*

Table A. Summary of morphometric data (±µm) of the observed *Monocystis eutyphae* sp. nov. stages in the seminal vesicles of *Eutyphoeus orientalis*

Parameters	Range	Mean	Median	SD	SE	CV%
Length of the trophozoite	100.7-107.8	104.83	104.39	2.02	0.45	1.92
Width of the trophozoite	51.93-59.67	55.68	55.23	3.13	0.69	5.62
Diameter of the nucleus	10.5-11.0	9.0	9.25	1.05	0.23	11.66
Length of the gametocyst	89.50-96.13	93.04	93.0	2.81	0.62	3.02
Width of the gametocyst	67.40-78.45	72.37	72.01	4.35	0.97	6.01
Length of the oocyst	13.25-15.47	14.30	14.02	0.91	0.20	6.36
Width of the oocyst	9.94-13.26	11.70	11.30	1.40	0.31	11.96

Table 1. The morphometric comparisons of *Monocystis eutyphae* sp. nov. (Present work) with *M. lalbagensis* and *M. nadiensis*

Species characters	***Monocysts lalbagensis*** (Bandyopadhyay *et.al., 2001)*	***Monocystis nadiensis*** (Bandyopadhyay *et.al., 2002)*	***Monocystis eutyphae*** sp. nov. (present work)
Host	*Metaphire posthuma*	*Metaphire posthuma*	*Eutyphoeus orientalis*
Locality	Lalbag, Murshidabad, India	Fulia, Nadia, West Bengal, India	Ashoknagar, 24 parganas (N), W.B., India
Site of infection	Seminal vesicles	Seminal vesicles	Seminal vesicles
Trophozoite	Cone shaped. Pellicle distinct, very delicate, length measures 62.4-108.1 µm and width measures 33.2 -53.2µm.	Ballon shaped, measuring 110.7-132.12µm x 74.99-85.70µm.	Fusiform, anterior part wide and posterior part is blunt. Total length 100.7-107.8 (104.83±2.02) µm. anterior portion width is 51.93-59.67 (55.68±3.13) µm and posterior portion width is 20.99-23.86 (22.69±1.08)µm.
Ectosarc	Not stated	Smooth	Smooth and irregular
Endosarc	Vacuoles excavated the endoplasm	Densely granulated	Granulated with paraglycogen granules
Nucleus	Spherical , measures 8.3 – 12.5 µm in diameter with an average of 9.3	Rounded, measures 10.7-14.2 (13.05±1.59)µm	Rounded, measures 10.5-11.0 (9.0±1.05) µm in diameter
Gametocyst	Ovoidal contains two unequal gametocytes. Larger one measuring 71.0-83.2 µm x 45.0-76.1µm and smaller one measuring, 58.2-75.0 x 32.1-50.0µm.	Ovoidal possess two unequal gametocytes. Larger one measuring 99.98-117.84 µm x 74.99-99.98µm and smaller one measuring 82.13-96.41µm x 74.99-82.33µm.	Ovoidal having two uneual gametocytes. It measures 89.50-96.3 (93.04±2.81) µm x 67.40-78.45 (72.37±4.35)µm.
Oocyst	Biconical, measuring 6.4-6.6 µm x 3.1-3.3µm in diameter.	Biconical, measures 8.3-9.7µm x 4.1-5.5µm in diameter.	Biconical, length measures13.26-15.47 (14.30-0.91) µm and width measures 9.94-13.26(11.70±1.40) µm.

Remark

The species under discussion shows similarities and dissimilarities with *Monocystis lalbagensis* and *Monocystis nadiensis* respectively. The present species differs from *M. Lalbagensis* relating its shape and size of the body. Trophozoites are cone shaped in M. lalbagensis, while they are balloon shaped in *M. nadiensis* and fusiform in *M eutyphae* sp. nov. In *M. lalbagensis* vacuoles excavate the endosarc whereas, in *M. nadiensis*, cytoplasm is densely granulated as like as that of present species. The anterior end of present species is wider than the posterior end which looks like blunt shaped. A big ovoidal distinct mucron is present in the anterior portion. The nucleus is rounded, present near the mucron at anterior portion of the body. Pellicle is thin. In the present form syzygy stage is present in the life cycle, while this stage is absent in the earlier described species. The position, shape and size of the nucleus, gametocyst and oocyst, host and the geographic location of the present species differ from the earlier described species (Table 1).

Taking into consideration all the differences with the closely related species, the present species seems to be new to science. Hence the name *Monocystis eutyphae* sp. nov. is being proposed here.

Monocystis *indicus* sp. nov. Figures 3(A-D), 4(A-D) Table B & Table 2

Description

In *Monocystis indicus* sp. nov. gamonts are oval shaped. Rounded nucleus is present at middle region of the body. Presence of smooth, thin and irregular shaped ectosarc. It contains highly granulated endosarc. At the anterior end of the body spherical mucron is present. Syzygy stage

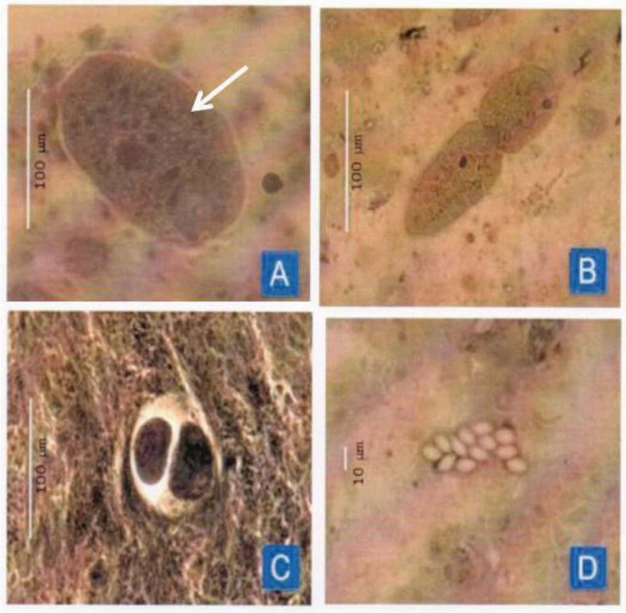

Figure 3. *Monocystis indicus* sp. nov. (Photomicrographs of different stages of life cycle) **A.** Trophozoite (white arrow – granulated endosarc). **B.** Syzygy. **C.** Gametocyst. **D.** Oocyst. Scales: A-C = 100µm, D = 10µm.

present in the life cycle. Gametocyst contains two unequal gametocytes and almost rounded in shape. Oocyst are biconical.

Gamont measures 89.61-111.6 (102.95±9.69) µm. x 59.29-70.72 (66.20±4.33) µm. Nucleus measures 14.30-20.4 (18.50±2.00) µm in diameter. Gametocyst measures 90.25-102.50 (39.25±5.55) µm in diameter. Large gametocyte measures 66.50-75.50 (70.90±4.50) µm x 35.80-45.50 (40.50±3.25) µm and small gametocyte measures 60.40-65.20 (64.54±3.54) µm x25.50-35.28 (31.50±2.50) µm. Oocyst measures 10.55-11.00 (10.77± 0.46) µm x 7.00-8.50 (6.0± 0.50) µm.

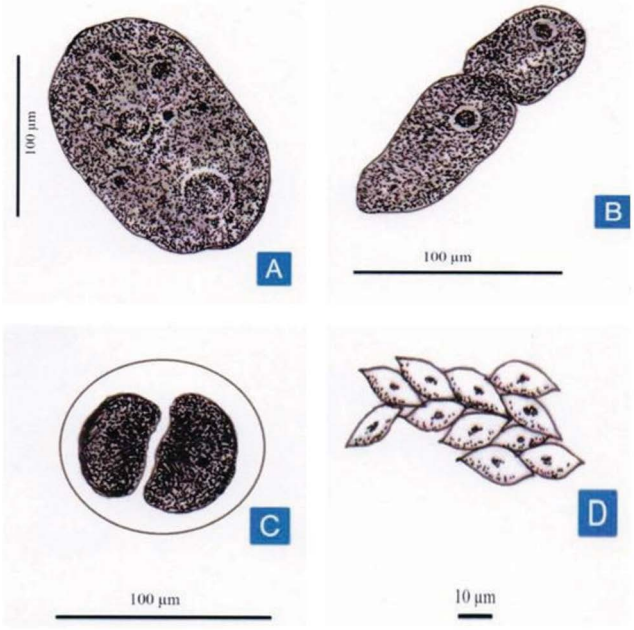

Figure 4. *Monocystis indicus* sp. nov. (Camera lucida drawings of different stages of life cycle) **A.** Trophozoite. **B.** Syzygy. **C.** Gametocyst. **D.** Oocyst. Scales: A-C =100µm; D = 10µm.

Taxonomic Summary

Type host: *Eutyphoeus orientalis*
Site of infection: Seminal vesicles
Type locality: Ashoknagar, North 24 Parganas (23°15'2"N-89°5'E), West Bengal, India.
Prevalence : 15 out of 40 host earthworms (37.5%) were infested with the parasite.

Material deposited

Holotype: PR/MC/03/12 has been deposited in the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Paratype: Systematic Research Collection, University of Nebraska State Museum, W436.Nebraska Hall.Lincoin, Nebraska 68588-0514 USA (Acession no. P-2014-002; Reg. no. HWML 49896).

Etymology: The species names have been given after the name of the nation India.

Table B. Summary of morphometric data (±µm) of *Monocystis indicus* sp. nov. stages in the seminal vesicles of *Eutyphoeus orientalis*

Parameters	Range	Mean	Median	SD	SE	CV%
Length of the trophozoite	89.61-111.6	102.95	102.51	9.69	2.16	9.41
Width of the trophozoite	59.29-70.72	66.20	66.03	4.33	0.96	6.54
Diameter of the nucleus	14.30-20.4	18.50	18.12	2.00	0.44	10.81
Length of the gametocyst	90.25-102.50	39.25	39.0	5.55	1.24	14.14
Length of the oocyst	10.55-11.00	10.77	10.33	0.46	0.10	4.27
Width of the oocyst	7.00-8.50	6.0	6.29	0.50	0.11	8.33

Table 2. The comparative studies of *Monocystis indicus* sp. nov. (Present work) with *M. ayeshae* and *M. arabindae*

Species characters	***Monocystis ayeshae*** (Sarkar and Bandyopadhyay 2010)	***Monocystis arabindae*** (Bandyopadhyay *et al.*, 2007)	***Monocystis indicus*** sp. nov. (present work)
Host	*Metaphire posthuma*	*Eutyphoeus incommodus*	*Eutyphoeus orientalis*
Locality	Satkhira , Bangladesh	West Midnapore	Ashoknagar, North 24Prganas, West Bengal, India
Site of infection	Seminal vesicles	Seminal vesicles	Seminal vesicles
Trophozoite	Oval shaped, contains extended middle portion. It measuring 92.82-97.24 (95.21±1.98)µm x 50.83-57.46 (53.40±3.24) µm	Solitary, cylindrical. Curved due to presence of constriction at one lateral surface. Measuring 127.0-145.5(137.1 ± 5.2) x 45.1-53.3(49.3 ± 2.6) µm	Oval potato like and blunt at both ends with extended middle part. It measures 89.61-111.6(102.95±9.69) µm x 59.29 -70.72 (66.20± 4.33) µm
Ectosarc	Thin and Smooth	Granular	Thin , smooth but irregular in shape
Endosarc	Granulated devoid of any spot	Vacuolated	Around the endosarc big highly granulated spot present
Mucron	Absent	Absent	Spherical, present at the anterior portion of the body and length measuring 20.50-24.25 (21.50 ±1 .19) µm and width measuring 18.50 – 20.80 (19.75 ± 0.92) µm
Syzygy	Absent	Absent	Syzygy present , the total length of syzygy is 250- 275 (201.25± 8.00) µm
Nucleus	Ovoidal, measures 13.26-17.68(16.02±1.91)µm in diameter	Rounded and measures 14.3-20.5(17.3 ± 1.9) x 12.3-18.9(16.1 ±1.1) µm	Nucleus rounded, present at the middle of the body. It measuring 14.30- 20.4 (18.50 ± 2.00) µm in diameter.
Gametocyst	Rounded measuring 132.6-141.44 (136.83±4.04) µm in length and measuring 7.24-101.66 (99.26±1.98) µm in width.	Ellipsodial and measures 79.9-96.3 (89.1 ± 5.0) x 69.7-84.0 (76.2 ± 4.6) µm	Gametocyst almost rounded with two unequal gametocytes, Gametocyst measures 90.25-102.50 (39.25±5.55) µm in diameter.
Oocyst	Navicular, measuring 8.84 – 15.47 (11.78±2.17) µm x 4.42 – 8.84 (6.81±1.75)µm in diameter.	Biconical measuring 8.0-13.9 (11.9±1.1)µm	Biconical, measures 10.55-11.00 (10.77 ±0.46) µm in length and measures 7.00-8.50 (6.0 ± 0.50)µm in width.

Remark

While comparing the present species with earlier described species *Monocystis ayeshae* and *M. arabindae*, it appears that the gamont of *M. ayeshae* is oval shaped bearing swolen middle part as that of the present species and in *Monocystis arabindae* the gamont is solitary, cylindrical curved due to presence of constriction at the lateral surface. In present species, mucron is prominent while in *M. ayeshae* and *M. arabindae* it is absent. In *M. ayeshae,* ectosarc is smooth, thin and granulated endosarc devoid of any spots. Nucleus is large and oval shaped. Whereas, ectosarc is smooth and thin in present species and highly granulated endosarc contains large granules. Nucleus is rounded and present at the middle portion of the body. In *M. arabindae* ectosarc is granulated and vaculated endosarc, nucleus rounded. The gametocyst of *M. ayeshae* is almost rounded contains unequal gametocytes as that of present species. While in M. arabindae gametocyst is ellipsoidal. In *M. ayeshae*, oocyst is navicular with pointed ends while it is biconical in *M.arabindae* as that of present form. Syzygy stage found in present form. While it is not present in *M. ayeshae* and *M. arabindae*. Moreover, *M. ayeshae* and *M. arabindae* have been reported from *Metaphire posthuma* of Satkhira, Bangladesh and *Eutyphoeus incommodus* from West Midnapore, West Bengal, India respectively but the present species have been described from the *Eutypohoeus orientalis* collected from Ashoknagar, West Bengal, India (Table 2).

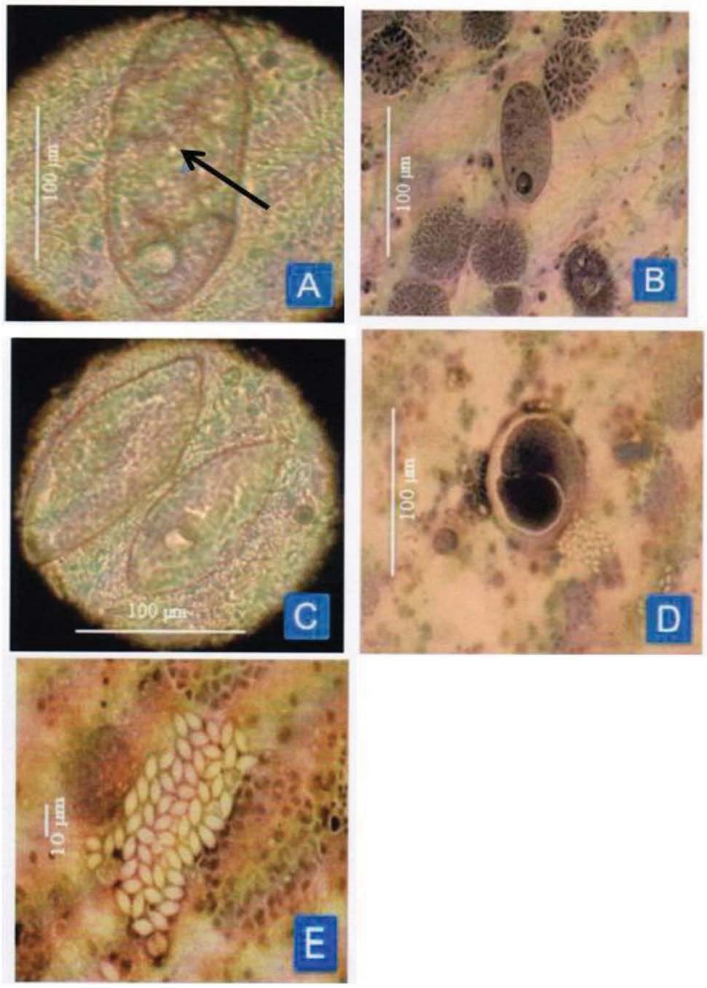

Figure 5. *Monocystis satoi* sp. nov. (Photomicrographs of different stages of life cycle)
A-B. Trophozoite (black arrow- vacuolated endosarc). **C.** Syzygy **D.** Gametocyst **E.** Oocyst.
Scales : A-D =100µm; E =10µm.

Considering all the differences with the closely related species the present species seems to be new to science. Hence the name *Monocystis indicus* sp. nov. is being proposed.

Monocystis satoi sp. nov. Figures 5(A-E), 6(A-E) Table C & Table 3

Description

Gamonts are elongated, flat and oval shaped. One end rounded and other cone shaped. Presence of smooth and thick ectosarc. Endosarc is vacuolated. Presence of distinct rounded nucleus at the conical portion of the gamonts. Gametocysts are rounded contains two unequal gametocytes. Oocysts are biconical having pointed ends.

The gamont measures 96.55-125.00 (15.25±8.25) x 50.28-62.50(59.22±5.00) µm. Nucleus measuring 12.35-14.50(13.75±1.00) µm in diameter. Gametocyst measures 70.00-75.00(72.00±4.32) µm in diameter. Oocyst measures 15.17-18.17(16.70±1.35) in length 9.75-11.25(10.00±1.00) µm in width.

Taxonomic Summary

Type host: *Eutyphoeus orientalis*
Site of infection: Seminal vesicles
Distribution: Ashoknagar, North 24 Parganas (23°15'2"N-89°5'E), West Bengal, India.

Prevalence: 10 out of 35 host earthworms (28.57%) were infested with the parasite.

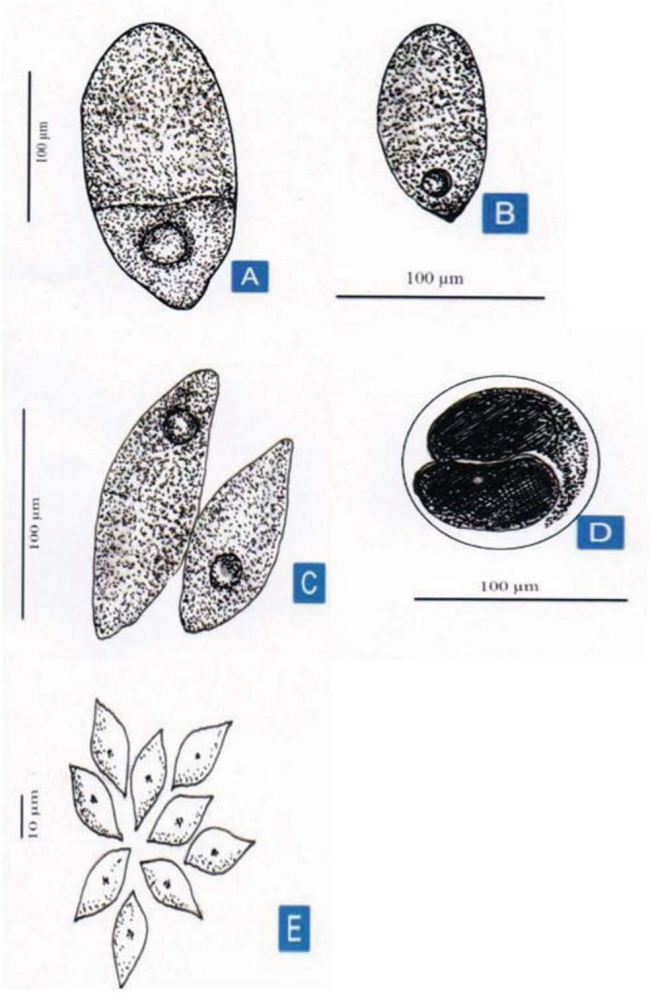

Figure 6. *Monocystis satoi* sp. nov. (Camera lucida drawings of different stages of life cycle)
A-B. Trophozoite. **C.** Syzygy **D.** Gametocyst. **E.** Oocyst. Scales : A-D =100µm; E=10µm.

Material deposited

Holotype: PR/MC/05/12 has been deposited at the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Paratype : The paratype has been deposited in the Harold W Manter, Laboratory of Parasitology, Systematic Research Collections, University of Nebraska State Museum, W436, Nebraska Hall, Lincoln, Nebraska 68588-0514 USA, (Accession no. P-2014-002; Reg. no. HWML 49896).

Etymology: The species name have been given after the name of renowned parasitologist Prof. Hiroshi Sato, Joint Faculty of Veterinary Medicine of Yamaguchi University, 1677-1 Yoshida, Yamaguchi, 753-8515, Japan for his outstanding contribution in the field of Parasitology.

Remark

While compairing, the present species with *Monocystis nidata*, *Monocystis pontodrilli* and *Monocystis darjeelingensis*,

Table C. Summary of morphometric data of *Monocystis satoi* sp. nov. stages in the seminal vesicles of *Eutyphoeus orientalis*

Parameters	Range	Mean	Median	SD	SE	CV%
Length of the trophozoite	96.55-125.00	15.25	15.24	8.25	1.84	54.09
Width of the trophozoite	50.28-62.50	59.22	59.19	5.00	1.11	8.44
Diameter of the nucleus	12.35-14.50	13.75	13.27	1.00	0.22	7.27
Length of the gametocyst	70.00-75.00	72.00	72.18	4.32	0.96	6
Length of the oocyst	15.17-18.17	16.70	16.40	1.35	0.30	8.08
Width of the oocyst	9.75-11.25	10.00	10.34	1.00	0.22	10

Table 3. The morphometric comparison of *Monocystis satoi* sp. nov. (Present work) with some closely related species

Species character	***Monoystis nidata*** (Boisson, 1957)	***Monocystis pontodrilli*** (Subbarao *et al*., 1979)	***Monocystis darjeelingensis*** (Bandyopadhyay and Mitra 2005)	***Monocystis satoi*** sp. nov. (Present work)
Host	*Pontodrilus ephippiger*	*Pontrodrilus barmudensis*	*Amynthas robusta*	*Eutyphoeus orientalis*
Locality	Asia, Indo-China	South India	Darjeeling, West Bengal, India	Ashoknagar , North 24 Pargnas, West Bengal, India
Site of infection	Seminal vesicles	Coelom, body muscle and Seminal vesicles	Coelomic fluid	Seminal vesicles
Trophozoite	Small cell like, elongated length 260-290 µm and width 90-120 µm	Elongated, measures 350µm x 150 µm.	Cylindroid having round extremities. One end slightly wider than the other. Conical or elliptical in young stage measuring 204- 273 µm x 84-140 µm.	Flat, elongated and oval shaped, measuring 96.55-125.00 (15.25 ±8.25) µm x 50.28- 62.50 (59.22± 5.00) µm in diameter.
Ectosarc	Thin, smooth	Thin, smooth	With fine longitudinal striations	Thick, smooth
Endosarc	Vacuolated	Vacuolated	Granulated	Vacuolated
Nucleus	Spherical situated in the center, 50 µm in diameter	Oval, position is variable	Spherical 8.3-12.5 µm in diameter.	Rounded with unequal gametocytes. It measures 70.00 – 75.00 (72.00 ± 4.32) in diameter.
Gametocyst	Not mentioned	Spherical, measures 350 µm x 160 µm in diameter.	Spherical 147- 163 µm	Rounded 12.35 – 14.50 (13.75 ±1.00) µm in diameter.
Oocyst	Navicular , 9 µm in length	Boat shaped, measuring 8.0 µm x 3.2 µm	Navicular, 15.4-18 µm x 8.0- 9.0 µm.	Biconical, measuring 15.17 –18.17(16.70±1.35) µm x 9.75–11.25(10.00± 1.00) µm.

it appears that the trophozoite is flat and oval shaped having one rounded and other conical end in the present form. Whereas in *M. nidata*, it looks like a small cell which possess two blunt ends, but in *M. pontodrilli* it is elongated having two broadly rounded ends. In *M. pontodrilli* the trophozoite crescent shaped and mucron is present in one end of the trophozoite. On the contrary, it is also crescent shaped in the present form. Nucleus is rounded and present in conical portion of the present species. But in *M. nidata* and *M. pontodrilli*, it is ovoid, centralized and rounded present either at the anterior-middle or the posterior end respectively. Trophozoite is cylindrical with rounded extremities of one end and slightly wider than other in *M. Darjeelingensis*. In *M. Darjeelingensis*, nucleus is spherical in shape and present in the narrow portion. Furthermore, *M. darjeelingensis* is much larger in all respects than the present form and earlier described other species. The oocyst is navicular in *M. nidata* and *M. darjeelingensis* respectively. While, in *M. Pontodrilli* it is spindle or boat shaped but biconical in the current species. Moreover, *M. nidata* and *M. pontodrilli* have been described from *Pontodrilus ephippiger* of Asia and Indo-China and *Pontodrilus barmudensis* of South India respectively whereas, *M. darjeelingensis* and the present species have been reported from *Amynthas robusta* of Senchal lake of Darjeeling and *Eutyphoeus orientalis* of Ashoknagar, North 24 Parganas district of West Bengal, India respectively.

Taking into account all the differences with the closely related species, the present species seems to be new one. Hence the name *Monocystis satoi* sp. nov. is given here.

Monocystis csabai sp. nov. Figures 7(A-D), 8(A-D) Table D & Table 4

Description

The trophozoite is elongated, irregular flat ribbon shaped contains tapering pointed end. Ectosarc is thick and the endosarc is vacuolated. Large, rounded very distinct nucleus is present at the middle portion of the body. Syzygy is present. In the life cycle of the present form two unequal overlapping almost rounded gametocyte have been found. Oocyst is biconical with pointed ends.

The trophozoite measures 132.0-160.0(145.0±4.50) x 30.0-35.0(32.2±2.50) µm. Nucleus measuring 15.5-

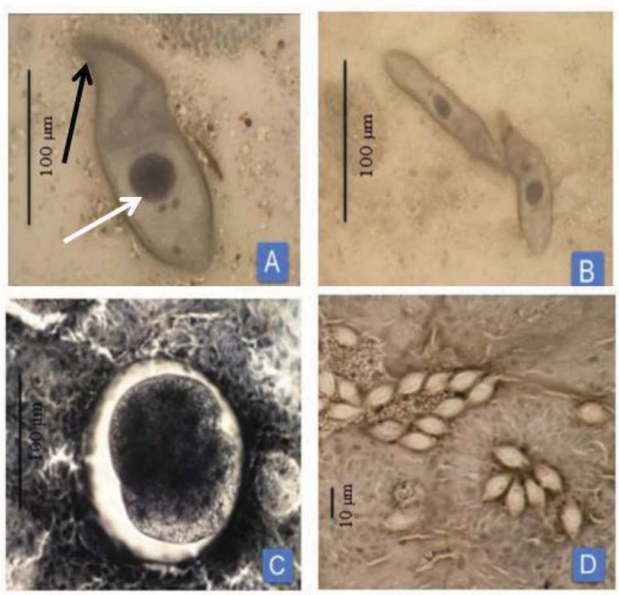

Figure 7. *Monocystis csabai* sp. nov. (Photomicrographs of different stages of life cycle) **A.** Trophozoite (white arrow- large rounded distinct nucleus); (black arrow- tapering pointed end). **B.** Syzygy. **C.** Gametocyst **D.** Oocyst. Scales: A-C = 100µm; D = 10µm.

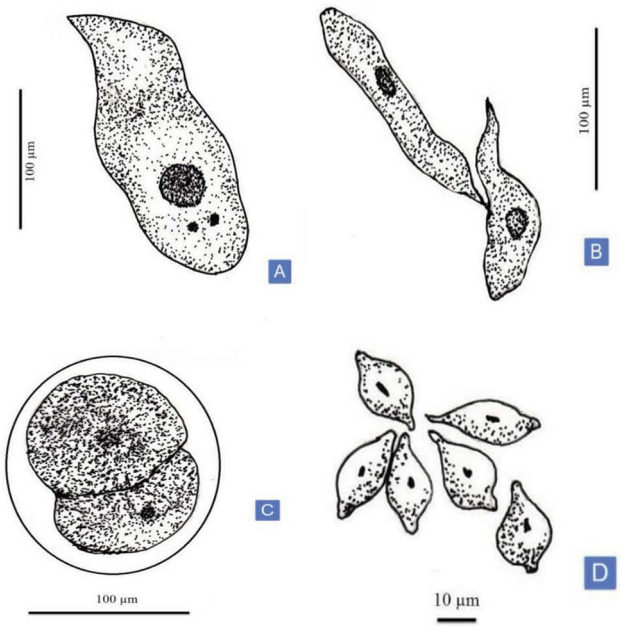

Figure 8. *Monocystis csabai* sp. nov. (Camera lucida drawings of different stages of life cycle) **A.** Trophozoite. **B.** Syzygy. **C.** Gametocyst **D.** Oocyst. Scales: A-C = 100µm; D = 10µm.

20.0(17.2±1.5) µm in diameter. Gametocyst measures 60.0-70.0.5(65.0±2.50) µm. Oocyst measures 9.0-12.5 (11.0±1.5) x 6.5-7.5(7.0±0.96) µm.

Taxonomic Summary

Type host: Eutyphoeus orientalis

Site of infection: Seminal vesicles

Distribution: Bakkhali (Sundarban) region, South 24 parganas (22.1352ºN, 88.4016ºE), West Bengal, India

Prevalence: 10 out of 25 host earthworms (40%) were infested with the parasite.

Material deposited

Holotype: PR/MC/34/12 has been deposited at the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani-741235, Nadia, West Bengal, India.

Paratype: The paratype has been deposited in the Harold WManter, Laboratory Parasitology, Systematic Research Collection University of Nebraska State Museum W 436, Nebraska Hall, Lincoin, Nebraska 68588-0514 USA 4 (Accession no. P-2014-002: Reg. no. HWML 49895).

Etymology: The species name have been given after the name of renowned parasitologist Prof. Csaba Szekely of Institute for Veterinary Medical Research, Centre for Agriculture Research, Hungarian Academy of Sciences, Budapest Hungaria Krt.21 for his outstanding contribution in the field of fish parasitology.

Table D. Summary of morphometric data (±µm) of *Monocysis csabai* sp. nov. in the seminal vesicles of *Eutyphoeus orientalis*

Parameters	Range	Mean	Median	SD	SE	CV%
Length of the trophozoite	132.0-160.0	145.0	145.19	4.50	1.00	3.10
Width of the trophozoite	30.0-35.0	32.2	32.26	2.50	0.55	7.76
Diameter of the nucleus	15.5-20.0	17.2	17.38	1.5	0.33	8.72
Dameter of the gametocyst	60.0-70.0	65.0	65.12	2.50	0.55	3.84
Length of the oocyst	9.0-12.5	11.0	11.25	1.50	0.33	13.63
Width of the oocyst	6.5-7.5	7.0	7.34	0.96	0.21	13.71

Table 4. The comparative studies of *Monocystis csabai* sp. nov. (Present work) with *Monocystis apporectodae* and *Monocystis elogngatum*

Species characters	***Monocystis apporectodae*** (Bandyopadhyay *et al*., 2006)	***Monocystis elongatum*** Bandyopadhyay *et al*., 2008	***Monocystis csabai*** sp. nov. (present work)
Host	*Apporectodea trapezoides*	*Perionyx excavatus* Perrier, 1872	*Eutyphoeus orientalis*
Locality	Bankura, West Bengal, India	Murshidabad, West Bengal	Bakkhali, South 24 Parganas, West Bengal, India
Site of infection	Seminal vesicles	Seminal Vesicles	Bakkhali, South 24 Parganas, West Bengal, India
Trophozoite	Elongated, irregular, flat, both side pointed, It measures 178.0- 224.0 (203.5±5.0) µm × 37.0- 58.0 (46.0±1.5)µm	Elongated and curved body, it measures 265.0-346.0 (310.0 ± 2.6) x 46.0 - 64.3 (52.0 ± 3.1)µm.	Elongated, irregular, flat, ribbon like contains gradually tapering pointed end flame like appearance measuring 132.0–160.0 (145.0 ± 4.50)µm × 30.0– 35.0 (32.2 ± 2.50)µm
Ectosarc	Thin	smooth	Thick
Endosarc	Densely granules	Highly granular and vacuolated with a large number of paraglycon granules.	Vacuolated

Nucleus	Ovoid contains densely packed karyosome, It measures 12.0- 16.0 (15.0±0.59) µm in diameter	Rounded to ovoid nucleus, measures 12.3-20.5 (19.4 ± 1.7) x 11.4-18.7(16.3 ± 1.8)	Large, rounded, very distinct, situated at middle portion of the trophozoite, measuring 15.5–20.0 (17.2 ±1.5) µm in diameter
Gametocyst	Ovoid. It measures 108.0-118.0 (113.0±1.1)µm × 79.0-89.0 (83.0±1.1)µm	Ovoid with two equal gametocytes, measuring 74.0-98.3 (82.0 ± 1.9) X 56.0-82.0 (72.5 ± 2.7)	rounded contains two unequal, overlapping gametocytes, measuring 60.0–70.0 (65.0±2.50) µm in diameter
Oocyst	Navicular, it measures 10.0-14.6 (13.4±1.1) µm × 5.5-8.1 (7.7± 2.3) µm	Biconical, measuring 9.7-15.3 9(12.3 ± 3.1) x 6.8-10.3 (9.4 ± 1.1) µm	Biconical pointed ends, measures 9.0–12.5 (11.0±1.5) µm × 6.5–7.5 (7.0±0.96)µm

Remark

The present species under discussion compares with *Monocytsis apporectodae* and *Monocystis elongatum*. The species under discussion has elongated, irregular, flat and ribbon like trophozoite, appears gradually tapering pointed end which is flame like in appearance. Whereas, it is elongated, irregular, flat like having both side pointed ends in *M. apporectodae* and in *M. elongatum* it is elongated and curved. Large and rounded nucleus is present in the present form. But in *M. apporectodae*, it is ovoidal contains densely packed karyosome and in *M. elongatum* nucleus is rounded to ovoid. Ectosarc is thick in the present specis while, in *M. apporectodae*, it is thin and in *M. elongatum* it is smooth. The present form has vacuolated endoplasm whereas in earlier described species endoplasm is densely granulated. The syzygy stage is found in the life cycle of both species. Moreover, the size and shape of the gametocysts and oocysts of the current form are also different from the previously described species. Furthermore, the present form has been reported from *Eutyphoeus orientalis* of Bakkhali, South 24 Parganas district of West Bengal. Whereas *M. apporectodae* and *M. elongatum* have been reported from *Apporectodea trapezoids* of Bankura district of West Bengal and *Perionyx excavatus* of Murshidabad, West Bengal, India respectively.

Comparing all these aspects the present form is being considered as a new one. Hence the name *Monocystis csabai* sp. nov. has been designated.

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